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Original Article

Role of *Leptospira* spp. testing and ocular examination in horses with equine recurrent uveitis: A retrospective study of 63 horses

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Keywords: horse; uveitis; leptospirosis; titres; PCR

Summary

Background: There is little information on the correlation between equine recurrent uveitis (ERU) and leptospiral infection status as it relates to ocular examination findings and testing recommendations.

Objective: To evaluate the role of leptospiral testing in horses with ERU by correlating results to signalment, clinical findings and visual outcome.

Study design: Retrospective case series.

Methods: Records of horses presenting for ERU at NC State University Veterinary Health Complex (NCSU-VHC) between 2014 and 2019 were reviewed. Signalment, initial visual status, ocular examination findings, treatments and visual outcome were collected. Serum and aqueous humour (AH) leptospiral titres were assessed for six different leptospirosis serovars and polymerase chain reaction (PCR) testing. Goldmann-Whitmer (C) values were calculated.

Results: Records of 63 horses and 78 eyes with ERU were evaluated. Horses with a positive aqueous humour PCR were significantly younger in age (mean 9.25 ± 1.14) than PCR negative horses (mean 13.3 ± 0.95) ($p = 0.01$). A positive aqueous humour titre of any serotype was significantly correlated to blindness at presentation ($p = 0.04$). A positive serum titre was significantly correlated to presence of ocular posterior segment disease ($p = 0.01$). Positivity to *L. bratislava* in the serum correlated to posterior segment disease ($p = 0.04$) and AH positivity correlated to blindness at presentation ($p = 0.002$).

Main limitations: Limitations of this study include that it is a retrospective and information gathered relies on data included within medical records.

Conclusions: Positive leptospiral testing results were associated with younger horses and ocular posterior segment clinical disease. Horses with aqueous humour titres to any leptospiral serotype, especially *L. bratislava* serovar, were correlated to poor prognosis for vision.

Clinical relevance

- To educate owners and veterinarians about the correlation between ERU clinical signs and leptospiral infection status.
- To help owners and veterinarians understand the role of leptospiral testing and ERU outcome/prognosis.
- To make recommendations about testing for leptospirosis as it relates to ERU, the leading cause of blindness in horses.

Introduction

Equine recurrent uveitis (ERU) is one of the most significant equine ophthalmic diseases and is the foremost cause of blindness in the horse (Deeg, 2008; Faber et al., 2000; Fritz et al., 2014; Gerding & Gilger, 2016; Pearce et al., 2007; Polle et al., 2014; Regan et al., 2012; Sandmeyer et al., 2020; Verma & Stevenson, 2012). Autoimmune attack dominated by T-helper cells targeting retinal autoantigens has been shown to perpetrate the disease when triggered by a foreign antigen (Deeg, 2008; Deeg et al., 2006a,b; Fritz et al., 2014; Regan et al., 2012; Verma & Stevenson, 2012). Leptospirosis is linked to ERU flares and is a suspected trigger for ERU in the United States as well as other places around the globe (Fritz et al., 2014; Pearce et al., 2007; Polle et al., 2014; Regan et al., 2012; Sandmeyer et al., 2020; Verma & Stevenson, 2012; Wollanke et al., 2018). Age and breed have been previously linked to development of ERU, with some breeds such as the Appaloosa having clearly defined genetic loci associations (Fritz et al., 2014; Sandmeyer et al., 2020). One frustrating aspect of ERU is that the initial inciting factor can be eliminated but still have ongoing flares, directing to immune modulation as a necessity for treatment focus (Deeg, 2008; Deeg et al., 2006a,b; Verma & Stevenson, 2012). Current treatment methods for ERU involve immunosuppression commonly in the form of topical corticosteroids and oral non-steroidal anti-inflammatories (NSAIDs) to control acute uveitis and delay the onset of new flares (Gerding & Gilger, 2016; Verma & Stevenson, 2012). Unfortunately, many horses develop blindness despite aggressive treatment (Gerding & Gilger, 2016; Regan et al., 2012). More invasive management strategies including vitrectomy, cyclosporine implants and other novel treatment approaches such as gentamicin injection, mesenchymal stem cell therapy and immunosuppressive gene therapy are developing (Fischer et al., 2019; Saldinger et al., 2020; Schnabel et al., 2012; Sherman et al., 2017).

Despite the evidence that leptospirosis has been correlated to ERU, there are no clear guidelines regarding testing, treatment or prevention of leptospirosis as it relates to ERU (Deeg, 2008; Polle et al., 2014; Regan et al., 2012; Voelter et al., 2020; Wollanke et al., 2018). The rationale behind titre and PCR testing is that if there are *Leptospira* antibodies or DNA within the eye or serum, this would predispose the horse to ERU (Polle et al., 2014; Verma & Stevenson, 2012). The Goldmann-Witmer coefficient, or C value, has been recognised to estimate the amount of antibody production within the eye and is therefore considered a useful method to

correlate leptospiral status and significance of ERU flares (Gerding & Gilger, 2016; Gilger, 2018; Polle et al., 2014; Voelter et al., 2020). To the authors' knowledge, there have not been studies looking into the direct correlation between leptospiral titres, PCR and ocular examination findings in ERU horses. By investigating the relationship between leptospirosis infection status, ocular examination findings and visual outcome, recommendations can be made for testing and treating horses that are predisposed to ERU. The purpose of our study is to evaluate the role of leptospiral testing in horses with equine recurrent uveitis by correlating results to signalment, clinical findings and visual outcome.

Materials and methods

Inclusion criteria

Medical and communication records of 63 horses that were presented to the NCSU-VHC with suspected ERU between November 2014 and August 2019 were reviewed. To be included in this study, horses needed to have a complete ophthalmic examination performed by a boarded veterinary ophthalmologist (DACVO) and a diagnosis of ERU. ERU was diagnosed at the time of initial examination based on documented episodes of uveitis and ocular examination findings indicative of two or more past uveitis episodes (Gilger et al., 2008; Polle et al., 2014; Sandmeyer et al., 2020; Verma & Stevenson, 2012). Horses without a history of previous uveitis flares, full ophthalmic examinations or any *Leptospira* spp. testing were excluded.

Signalment data

The breed, age and sex of each horse were collected from medical records. Initial admission date of each horse to the NCSU-VHC and the date of the last visit or communication were recorded. Communication logs and medical records were analysed for owners' knowledge about the duration of ERU prior to the initial examination based on a previous diagnosis by a veterinarian, clinical signs of uveitis or signs of vision loss at home.

Ocular examination

Horses were examined by boarded veterinary ophthalmologists during the initial examination at NCSU-VHC. Both eyes of affected horses received a complete examination and findings were recorded. To help facilitate a complete examination, horses were typically sedated with intravenous detomidine (Dormosedan, Zoetis Inc., Parsippany, NJ, USA). Motor and sensory innervation from the auriculopalpebral and frontal nerves, respectively, were routinely denervated with approximately 1 ml of lidocaine HCL 2% (VEDCO Inc., Saint Joseph, MO, USA) injected subcutaneously. Intraocular pressures were measured with tonometry using either a Tonopen (Avia Vet, Reichert Inc., Depew, NY, USA) or Tonovet (Icare USA Inc., Raleigh, NC, USA). A slit lamp biomicroscope (KOWA SL-17) and indirect ophthalmoscopy (Keeler Vantage indirect, Keeler, Malvern, PA, USA) were used for ocular examination.

Vision status was determined by subjectively evaluating the horse's response to environmental stimuli, ability to navigate in its environment, dazzle reflex and the presence of a menace response. Pupillary light reflexes, both direct and consensual, were also assessed and recorded. The anterior segment was analysed for signs of active and chronic uveitis including aqueous flare graded on a scale of increasing severity (0, 0.5, 1, 2, 3, 4), depth of the anterior chamber, keratic precipitates, fibrin, hypopyon, hyphema, cells, synechiae and corpora nigra atrophy (Deeg, 2008; Deeg et al., 2006a; Hollingsworth & Gilger, 2016; Sandmeyer et al., 2020; Stoppini & Gilger, 2016). Aqueous flare score is a subjective grading scheme to assess for cells or protein in the anterior chamber. The lens was evaluated for the presence and location of cataract if present. The ocular posterior segment was evaluated for the presence of disease or inflammation. The posterior segment and presence of cataracts could not be evaluated in all cases due to anterior structure disease in some eyes. Changes in the vitreous often included cells, debris, degeneration, fibrin, haze and discoloration (Deeg et al., 2006a; Hollingsworth & Gilger, 2016; Sandmeyer et al., 2020). The optic nerve head (ONH) was assessed for cupping, pallor, and changes in size that could indicate damage. Retinal changes such as thinning, scarring, detachment and reflectivity were recorded (Deeg et al., 2006a; Sandmeyer et al., 2020).

Serum and aqueous humour testing for *Leptospira* titres and PCR

Serum was collected via venipuncture for *Leptospira* PCR and serum titres for six different serovars: *L. pomona*, *L. grippotyphosa*, *L. canicola*, *L. icterohaemorrhagiae*, *L. hardjo* and *L. bratislava* (**Fig 1**). Aqueous humour samples were collected via aqueocentesis for both leptospiral titre and PCR testing for the same serovars as tested for serology (**Fig 1**). Aqueocentesis was performed after sedation by inserting a 27 gauge needle at the dorsotemporal limbus into the anterior chamber (Stoppini & Gilger, 2016). Microagglutination testing (MAT) was performed to assess titres (Faber et al., 2000; Gilger et al., 2008; Verma & Stevenson, 2012; Voelter et al., 2020). For serum titres, a result of ≥1:400 was considered positive (Gerding & Gilger, 2016). The chosen titre cut-off is higher than some previous studies but was chosen to represent active infections of a particular *Leptospira* serovar rather than cross-reactivity between serovars. Results indicated if the PCR was negative or positive based on the presence of leptospiral DNA in the sample (Faber et al., 2000). Samples were analysed by the diagnostic lab at the NCSU-VHC. The C value was calculated for each serovar and recorded by determining the ratio of the aqueous humour titre to the serum titre (Faber et al., 2000; Gilger, 2018). In this study, a positive C value was considered ≥4 (Fischer et al., 2019; Gerding & Gilger, 2016).

Treatment

Initial treatments and treatments performed at recheck visits or based on follow-up communication were recorded (**Supplementary Item 1**). Horses that tested positive for leptospirosis, or if there was a severe flare of ERU, were usually placed on doxycycline or minocycline (Gilmour et al., 2005; Schnabel et al., 2012).

Visual outcome

Visual outcome of the affected eyes was determined based on ocular examination by a boarded veterinary ophthalmologist and owner follow-up. Eyes were classified to have good vision, decreased vision, or were blind. Blind eyes lacked menace or dazzle and often had signs of severe chronic damage from ERU. Eyes with decreased vision often

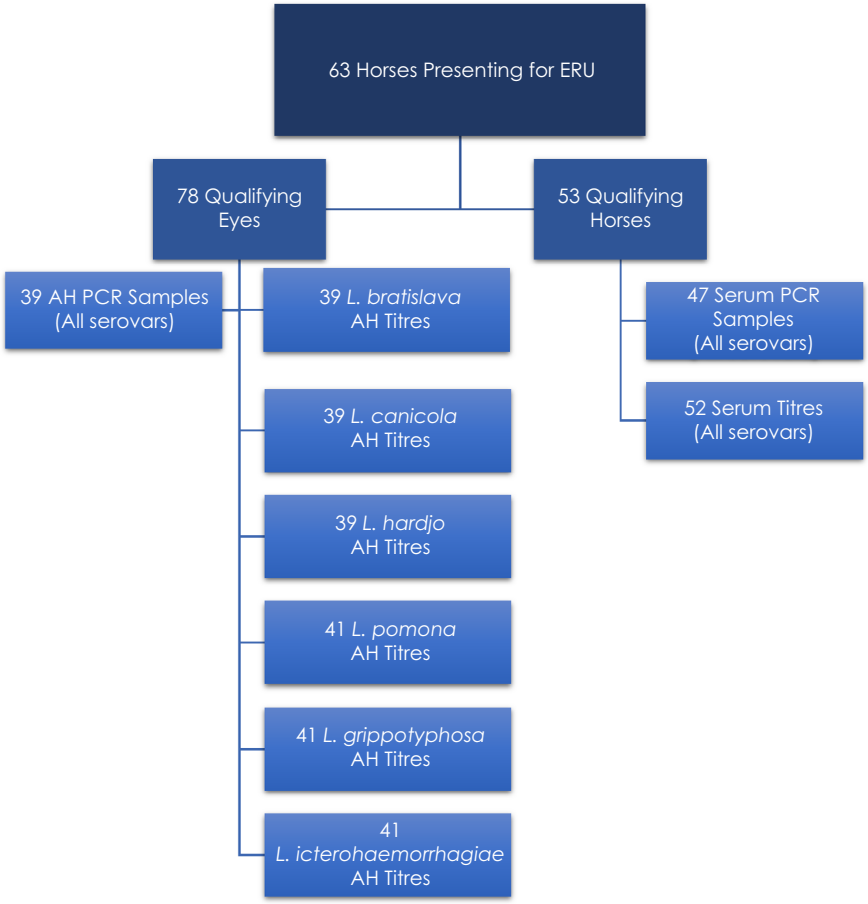

Fig 1: Flow diagram demonstrating the process for leptospiral testing and the number of available results for each corresponding test and serovar.

lacked a consistent menace response or had obvious anterior segment infiltrate that compromised the visual field. Eyes with good vision had normal PLRs, menace, dazzle and behaved visual despite any signs of uveitis. Communication logs were evaluated to determine the success of treatments and changes in vision status over time. The duration of follow-up from the last visit to the last communication either from the owner or referring veterinarian was recorded.

Data analysis

Data were analysed using JMP statistical software (SAS Inc.). Comparisons were made for parametric data using ANOVA, t-tests, and Tukey-Kramer tests. Non-parametric data were analysed using Fisher's exact test, Pearson chi-squared tests and likelihood ratios. Associations between and within groups were analysed. Age and follow-up time did not follow a normal distribution and are represented by mean and standard deviation. Categorical data are presented using proportions within the text. Data were considered significant if $P \leq 0.05$.

Results

General representation

Sixty-three horses presented to the NCSU-VHC for a suspected diagnosis of ERU. Breed and signalment data are listed in **Table 1**. Quarter Horses 17/63 (26.98%) and geldings 35/63 (55.56%) were most common, and the mean age at diagnosis of ERU was 11.3 years (mean 11.3 ± 5.36) (**Table 1**). Duration of a known ERU flare prior to initial examination ranged from 0.13 months to 60 months, with the average duration of 10.81 months. Four horses had a history of known trauma to the affected eye, one horse had a history of phacoemulsification surgery 2 years prior to presentation, and one horse was suspected to have developed uveitis after leptospiral vaccine administration. Out of the 63 horses that were presented to the NCSU-VHC for a suspected diagnosis of ERU, 53 were diagnosed with ERU based on complete ophthalmic examination and accessible *Leptospira* titres.

Vision status and ocular examination

Out of the 53 horses diagnosed with ERU and included in this study, 78 eyes were affected (**Table 1**). The mean follow-up time for visual outcome assessment from the time of the last visit was 3.46 months (mean 3.46 ± 6.06) with a range of 0 months of follow-up to 28 months. All eyes were assessed for anterior segment disease. Anterior segment clinical signs on examination were present in most eyes. In eyes that could be evaluated for posterior segment disease, 37/59 (62.71%) had disease present (**Supplementary Item 2**).

PCR testing of serum

Forty-seven horses had serum PCR testing performed for leptospiral DNA (**Fig 1**). Only 1/47 (2.13%) serum sample was

TABLE 1: Signalment data and vision status of horses presenting to the NCSU-VMC for suspected ERU organised by breed

Breed	Number of horses	Gender	Mean age (years)	Eyes affected	Vision of eyes at presentation	Vision of eyes at outcome
Andalusian	1 (1.59%)	MC (0/1) M (1/1) F (0/1)	25	OS (1/2) OD (1/2)	Present (2/2) Absent (0/2)	Present (2/2) Absent (0/2)
Appaloosa	7 (11.11%)	MC (5/7) M (0/7) F (2/7)	12.14	OS (6/12) OD (6/12)	Present (10/12) Absent (2/12)	Present (8/12) Absent (4/12)
Arabian	4 (6.35%)	MC (1/4) M (0/4) F (3/4)	13	OS (3/4) OD (1/4)	Present (3/4) Absent (1/4)	Present (3/4) Absent (1/4)
Connemara Pony	1 (1.59%)	MC (0/1) M (0/1) F (1/1)	17	OS (0/1) OD (1/1)	Present (1/1) Absent (0/1)	Present (0/1) Absent (1/1)
Fox Trotter	1 (1.59%)	MC (1/1) M (0/1) F (0/1)	17	OS (0/1) OD (1/1)	Present (1/1) Absent (0/1)	Present (1/1) Absent (0/1)
Friesian	1 (1.59%)	MC (0/1) M (0/1) F (1/1)	n/a	n/a	n/a	n/a
Hackney	1 (1.59%)	MC (1/1) M (0/1) F (0/1)	2	OS (1/2) OD (1/2)	Present (2/2) Absent (0/2)	Present (2/2) Absent (0/2)
Hanoverian	2 (3.18%)	MC (1/2) M (0/2) F (1/2)	12	OS (1/3) OD (2/3)	Present (3/3) Absent (0/3)	Present (2/3) Absent (1/3)
Irish Hunter Horse	1 (1.59%)	MC (1/1) M (0/1) F (0/1)	12	OS (1/2) OD (1/2)	Present (2/2) Absent (0/2)	Present (2/2) Absent (0/2)
Morgan	3 (4.76%)	MC (2/3) M (0/3) F (1/3)	12.67	OS (2/4) OD (2/4)	Present (2/4) Absent (2/4)	Present (3/4) Absent (1/4)
Mule	1 (1.59%)	MC (0/1) M (0/1) F (1/1)	10	OS (1/1) OD (0/1)	Present (1/1) Absent (0/1)	Present (1/1) Absent (0/1)
National Spotted Saddle Horse	1 (1.59%)	MC (1/1) M (0/1) F (0/1)	14	OS (1/2) OD (1/2)	Present (2/2) Absent (0/2)	Present (2/2) Absent (0/2)
Norwegian Fjord	1 (1.59%)	MC (0/1) M (0/1) F (1/1)	17	OS (0/1) OD (1/1)	Present (1/1) Absent (0/1)	Present (1/1) Absent (0/1)
Paint Horse	3 (4.76%)	MC (2/3) M (0/3) F (1/3)	10	OS (0/1) OD (1/1)	Present (1/1) Absent (0/1)	Present (1/1) Absent (0/1)
Paso Fino	1 (1.59%)	MC (0/1) M (0/1) F (1/1)	12	OS (1/2) OD (1/2)	Present (2/2) Absent (0/2)	Present (1/2) Absent (1/2)
Percheron	1 (1.59%)	MC (0/1) M (0/1) F (1/1)	n/a	n/a	n/a	n/a
Pony	1 (1.59%)	MC (0/1) M (1/1) F (0/1)	n/a	n/a	n/a	n/a
Quarter Horse	17 (26.98%)	MC (10/17) M (1/17) F(6/17)	9.5	OS (14/25) OD (11/25)	Present (18/25) Absent (7/25)	Present (17/25) Absent (8/25)
Saddlebred	1 (1.59%)	MC (1/1) M (0/1) F (0/1)	10	OS (1/1) OD (0/1)	Present (1/1) Absent (0/1)	Present (0/1) Absent (1/1)
Selle Francais Horse	2 (3.18%)	MC (1/1) M (0/1) F (1/1)	n/a	n/a	n/a	n/a
Thoroughbred	8 (12.70%)	MC (7/8) M (0/1) F (1/8)	12	OS (4/9) OD (5/9)	Present (6/9) Absent (3/9)	Present (5/9) Absent (4/9)

TABLE 1: Continued

Breed	Number of horses	Gender	Mean age (years)	Eyes affected	Vision of eyes at presentation	Vision of eyes at outcome
Walking horse	3 (4.76%)	MC (1/3) M (0/3) F (2/3)	8	OS (2/4) OD (2/4)	Present (4/4) Absent (0/4)	Present (4/4) Absent (0/4)
Warm Blood	1 (1.59%)	MC (0/1) M (0/1) F (1/1)	7	OS (1/1) OD (0/1)	Present (1/1) Absent (0/1)	Present (1/1) Absent (0/1)
Total	63 (100%)	MC (35/63 56%) M (3/63 5%) F (25/63 39%)	11.3	OS (40/78 51%) OD (38/78 49%)	Present (63/78 81%) Absent (15/78 19%)	Present (56/78 72%) Absent (22/78 28%)

Note: Horses that presented for ERU but did not meet the requirements for further consideration in this study are represented with n/a (i.e. no ERU, no past episodes of uveitis, incomplete ophthalmic exams, no leptospiral testing). Data collected upon initial admission are presented below and included breed (n = 23), gender, age, eye(s) affected and changes in vision status. Vision status is reported by eyes affected as some horses were diagnosed with ERU bilaterally. Vision was assessed during ophthalmic examination by a boarded ophthalmologist via menace, dazzle and ability to navigate through the environment. Visual outcome was assessed based on recheck examinations and owner follow-up.

positive on PCR for leptospiral DNA. The one positive serum PCR sample was from a 7-year-old Morgan Horse gelding and the corresponding AH PCR sample was negative.

Titre testing of serum

Serum titres for leptospirosis were performed on all 53 horses with ERU; however, complete results were not recorded in the medical record for one horse. This horse was treated for a positive leptospiral infection based on reported results. In 38/52 (73.08%) of horses tested with accessible results, a positive titre (≥1:400) to at least one serovar was found. A positive serum titre was significantly correlated to presence of ocular posterior segment disease ($p = 0.01$; Fisher's exact test) (**Table 2**).

PCR testing of aqueous humour

PCR testing of the aqueous humour was performed in 39/78 (50%) eyes. In 16/39 (41.03%) of eyes tested, a positive aqueous humour PCR test was found. Eyes with a positive aqueous humour PCR belonged to horses significantly younger in age (mean 9.3 ± 1.1 years) than PCR negative eyes (mean 13.3 ± 0.95 years) ($p = 0.01$; ANOVA) (**Table 2**).

Titre testing of aqueous humour

Thirty-nine eyes had AH titre testing performed for all serovars and 41 had testing for only *L. pomona*, *L. grippotyphosa* and *L. icterohaemorrhagiae* (**Fig 1**). Twenty-five eyes were positive to at least one leptospiral serovar. A positive aqueous humour titre to any serovar was correlated to blindness at presentation ($p = 0.04$; ANOVA) (**Table 2**). Eyes that had a positive titre to *L. bratislava* in the aqueous humour were highly correlated to vision loss at presentation ($p = 0.002$; Tukey-Kramer) and were either blind or had decreased vision at initial presentation (**Table 2**).

C value

The C value was calculated in 42/78 (53.85%) of eyes. In 22/42 (52.38%) eyes, a C value ≥4 was calculated, indicating a positive result for the purposes of our study. For 19/22 (86.36%) of positive eyes, the C value was positive for more than one serovar. The most common serovar to contribute to a positive C value was *L. grippotyphosa* 18/22 (81.82%) followed by *L. pomona* 15/22 (68.18%).

Leptospiral serovars

Positivity to *L. bratislava* serovar correlated to posterior segment disease ($p = 0.04$; ANOVA) as well as blindness ($p = 0.002$; Tukey-Kramer) and decreased vision ($p = 0.001$; ANOVA) at presentation. The serovar *L. grippotyphosa* ($p = 0.02$; ANOVA) was associated with blindness at presentation and serovar *L. hardjo* was associated with decreased vision at presentation ($p = 0.02$; ANOVA) based on a positive C value (**Table 2**). Positive serum titres generally corresponded to positive AH titres of the same serovar.

Treatments

All but one horse received treatment for ERU on the initial visit. In 41/53 (77.36%) of horses, secondary treatments were initiated at subsequent visits. Primary and secondary treatments are summarised in **Supplementary Item 1**.

Discussion

Leptospira infection is highly speculated to predispose horses to developing ERU, a devastating disease in the equine industry that leads to blindness. Leptospiral infection status and testing as they relate to ERU remain unclear. This study evaluated the role of leptospiral testing for six different serovars in horses with ERU using PCR and MAT testing of serum and aqueous humour. Despite treating active leptospiral infections, horses can still suffer from flares of ERU once *Leptospira* is cleared from the body (Deeg, 2008; Deeg et al., 2006a,b; Gilger, 2018; Gilger et al., 2008; Pearce et al., 2007; Regan et al., 2012; Verma & Stevenson, 2012). Prevention and recognition of *Leptospira* infection through ocular examination and appropriate testing can potentially preserve vision in horses by delaying ERU flares or preventing development of the disease. Our results demonstrated that leptospiral testing is correlated to age, clinical findings and visual outcome.

Surprisingly, *L. bratislava* was associated with a poor visual prognosis and the presence of active ocular disease. To our knowledge, *L. bratislava* has not been previously identified as a serovar directly connected to ERU flares in the southeast United States. Blindness at presentation was linked with *L. bratislava* and *L. grippotyphosa*, while *L. hardjo* was associated with decreased vision. Previous studies have

TABLE 2: Positive titre and PCR results with associated ocular examination findings and visual outcome organised by serovar

	L. pomona titre		*L. grippotyphosa* titre*		*L. icterohaemorrhagiae* titre		*L. hardjo* titre**		*L. bratislava* titre		All serovars PCR	
	AH	Serum	AH	Serum	AH	Serum	AH	Serum	AH[§]	Serum[‡]	AH[†]	Serum
Blind at presentation[§]												
(n positive eyes)	3	5	2	2	3	4	2	1	3	7	2	1
Per cent	7.3%	9.6%	4.9%	3.8%	7.3%	7.7%	5.1%	1.9%	7.7%	13.5%	5.1%	2.1%
Total samples	41	52	41	52	41	52	39	52	39	52	39	47
Blind at outcome												
(n positive eyes)	4	5	3	4	3	8	4	1	4	9	4	0
Per cent	9.8%	9.6%	7.3%	7.7%	7.3%	15.4%	10.3%	1.9%	10.3%	17.3%	10.3%	0.0%
Total samples	41	52	41	52	41	52	39	52	39	52	39	47
Anterior clinical signs												
(n positive eyes)	15	17	16	13	13	24	7	3	12	26	16	1
Per cent	36.6%	32.7%	39.0%	25.0%	31.7%	46.2%	17.9%	5.8%	30.8%	50.0%	41.0%	2.1%
Total samples	41	52	41	52	41	52	39	52	39	52	39	47
Posterior clinical signs[‡]												
(n positive eyes)	8	8	6	7	7	15	2	0	6	13	10	0
Per cent	19.5%	15.4%	14.6%	13.5%	17.1%	28.8%	5.1%	0.0%	15.4%	25.0%	25.6%	0.0%
Total samples	41	52	41	52	41	52	39	52	39	52	39	47

Titres were positive if results were ≥ 1:400 and eyes could be positive for more than one serovar. There were no positive titre results for *L. canicola*. Total sample numbers represent the number of results where specific serovar titre results were available. Visual status was determined by ophthalmic examination (i.e. presence of menace, dazzle, ability to navigate environment). All eyes were observed for anterior clinical signs (n = 78 eyes). Posterior clinical signs could be observed in only 59 eyes due to anterior disease. A positive aqueous humour PCR test was found in 16/39 (41.02%) of eyes tested and only one eye was correlated to a positive serum PCR. A positive aqueous humour titre was found in 25/41 (60.98%) of eyes tested and 38/52 (73.08%) of results correlated to a positive serum titre.

Abbreviations: AH, aqueous humour; PCR, polymerase chain reaction.

* A positive C value for *L. grippotyphosa* was associated with blindness at initial presentation ($p = 0.02$; ANOVA).

† Any positive AH PCR was associated with younger horses ($p = 0.01$; ANOVA).

‡ Any positive serum titre was associated with posterior segment disease ($p = 0.01$; Fisher's exact test), especially *L. bratislava* ($p = 0.04$; ANOVA).

§ Any positive AH titre was associated with blindness at presentation ($p = 0.04$; ANOVA), especially *L. bratislava* ($p = 0.002$; Tukey-Kramer). *L. bratislava* was also associated with decreased vision ($p = 0.001$; ANOVA).

** A positive C value for *L. hardjo* was associated with decreased vision status at initial presentation ($p = 0.02$; ANOVA).

correlated *L. bratislava* to a positive PCR in horses with uveitis, suggesting that this serovar initiates an immune response within the eye (Faber et al., 2000). Interestingly, *L. pomona* and *L. grippotyphosa* were previously reported to be common serovars responsible for ERU (Gilger et al., 2008; Polle et al., 2014; Verma & Stevenson, 2012). Our findings suggest that these serovars are still common, but there may be a stronger correlation between vision changes in ERU and certain serovars like *L. bratislava*, *L. grippotyphosa* and *L. hardjo*. Similarly, in the findings by Fischer et al., (2019), *L. grippotyphosa* was associated with disease or a positive C value (Gerding & Gilger, 2016; Gilger et al., 2008; Voelter et al., 2020). In a study by Malalana et al., (2017), *L. hardjo* was associated with a positive C value. Another explanation for these findings includes potential cross-reactivity between leptospiral serovars (Hollingsworth & Gilger, 2016; Yan et al., 2010). In the study by Yan et al., (2010), it was suggested that repeat titre testing may reveal the true infecting serovar.

The C value represents the correlation between serum and AH titres. A larger C value was used to account for seropositivity of various *Leptospira* serovars in the general horse population. The blood-ocular barrier is jeopardised with inflammation and infectious agents in blood can pass into the eye in the face of inflammation. This may also explain why any positive serum titre was related to posterior and anterior segment disease. Over half (53%) of the eyes that had posterior segment disease were positive to at least one *Leptospira* serovar in the correlating serum titre and many were positive to more than one serovar. The one positive serum PCR result in this study despite positive AH samples supports the thought that *Leptospira* is cleared from systemic circulation and sustained presence of the bacteria within the eye is not required for ERU flares. The understanding of the pathogenesis and virulence of different serovars could provide more effective ways at preventing infection. Based on our findings, serovar differentiation may be useful as a prognostic indicator for vision in horses with ERU.

Horses with aqueous humour titres to any leptospiral serotype, especially the *L. bratislava* serovar, were correlated to poor prognosis for vision. This is a similar finding to the study by Polle et al., (2014) where horses with uveitis had positive aqueous humour PCR and MAT results. A high number of aqueous humour antibodies and autoantigens suggest a larger scale of immune-mediated attack and secondary damage (Deeg, 2008; Faber et al., 2000). A plausible explanation is that leptospiral antigens within the eye are targeted by immune cells causing intraocular damage leading to blindness. In some horses, secondary damage within the eye leads to vision-threatening complications like glaucoma. Unsurprisingly, more eyes were considered to have good vision at presentation and there was an increase in the number of blind eyes at outcome.

Age of horse was correlated to a positive aqueous humour PCR for *Leptospira* DNA. The average age of an aqueous humour PCR positive horse was 9.25 years, which is younger than some of the previously reported common age of ERU-affected horses (Gilger, 2018; Polle et al., 2014; Sandmeyer et al., 2020). This suggests that horses between the ages of 6.5 and 12 years with signs of ERU should have aqueous humour PCR samples drawn to have the highest success of diagnosing *Leptospira* as an inciting factor. Based on our findings, the best chance of obtaining a positive aqueous humour PCR is likely when horses are in the acute phase of disease as bacteria are more likely to cross the blood-ocular barrier with active inflammation. Very young horses may not yet have clinical signs of ERU because it is thought that immune modulation and epitope spreading are responsible for the cyclical nature of ERU (Deeg, 2008; Deeg et al., 2006a,b; Pearce et al., 2007). Older horses may clear the leptospiral organisms in the serum and aqueous humour, but the theory of epitope spreading and immune modulation suggest that primed T cells stay locally within the eye for more severe and subsequent flare-ups (Deeg, 2008; Deeg et al., 2006a; Pearce et al., 2007; Verma & Stevenson, 2012; Voelter et al., 2020). Immune shifts targeted at autoantigens such as interphotoreceptor-retinoid binding protein (IRBP) and cellular retinaldehyde-binding protein (cRALBP) may contribute to subsequent ERU flares (Deeg, 2008; Deeg et al., 2006a,b; Regan et al., 2012; Verma & Stevenson, 2012). Age is reported to be a contributing factor for development of uveitis in Appaloosas (Sandmeyer et al., 2020).

There were no significant differences between breed and incidence of ERU when compared to the general equine ophthalmology population within the study timeframe. The power of our study was likely not high enough to draw conclusions based on breed and ERU incidence. Although not supported by our data, Appaloosas were previously overrepresented with ERU and are eight times more likely to develop uveitis compared to other breeds (Fritz et al., 2014; Gerding & Gilger, 2016; Sandmeyer et al., 2020). A recent study in Belgium positively correlated intraocular leptospirosis and ERU incidence in Appaloosas and Warmbloods (Sauvage et al., 2018). These horses often have insidious disease and the leptospiral testing window may be missed altogether (Fritz et al., 2014; Sandmeyer et al., 2020). Certain breeds in this study such as Hanoverians, had a 100% positive C value rate although not statistically significant. This is interesting as Hanoverians have been reported to be another overrepresented breed in ERU (Gerding & Gilger, 2016). There was not a strong association between breed and visual outcome; however, this has been previously reported in Appaloosas where affected horses were four times more likely to go blind (Fritz et al., 2014; Gerding & Gilger, 2016; Sandmeyer et al., 2020). Based on previous studies, certain breeds like Appaloosas may benefit from more frequent screenings for ERU and leptospirosis infection status (Verma & Stevenson, 2012). Another explanation to varied incidence of ERU around the world is genetic makeup of horses (Malalana et al., 2017). The MHC class variation in individual horses within breeds account for differences in uveitis flares, making it even more challenging to understand the specific role of leptospirosis in ERU (Deeg et al., 2006a; Regan et al., 2012; Sandmeyer et al., 2020). There was not a significant relationship between ERU and sex of horse or which eye was affected, similar to previous findings (Faber et al., 2000; Polle et al., 2014; Sandmeyer et al., 2020).

Serovar prevalence varies based on environment and season in other parts of the world (Vera et al., 2019; Verma & Stevenson, 2012; Voelter et al., 2020). In a study on leptospiral serovar prevalence in the United Kingdom, it was concluded that some serovars may be responsible for ERU, especially *L. sejroe* which was not tested for in this study (Matthews et al., 1987). More recent studies performed in the UK and Italy demonstrated that *L. bratislava* was the most common serovar detected in serum positive horses (Malalana et al., 2017; Vera et al., 2019). In Switzerland, *L. grippotyphosa* has

been reported as the most common serovar responsible for leptospiral-induced ERU (Tömördy et al., 2010). Most horses in this study resided in North Carolina and the southeast United States. The serovar prevalence is suspected to change based on climate so some conclusions drawn from this study may vary based on location (Voelter et al., 2020). *Leptospira* is known to have seasonal peaks of infection, which should be considered when recommending vaccines and preventative environmental measures. This is especially true in the southeast United States where natural disasters like floods and hurricanes are common (Verma & Stevenson, 2012). *Leptospira* prefers wet seasons and it may take months for ERU to develop after months with high precipitation (Verma & Stevenson, 2012).

Early detection of *Leptospira* infection of horses may be the most effective way of preventing ERU as many treatments fail (Verma & Stevenson, 2012). Testing horses for leptospiral organisms in urine with PCR can reduce exposure to other horses and environmental contamination (Gilger, 2018). It is also recommended to limit the exposure or frequently test horses with access to standing water, cattle, rodents, deer and other wildlife (Vera et al., 2019; Verma & Stevenson, 2012). The recently developed SNAP test for *Leptospira* may be of use in cases where there is a high suspicion of leptospirosis, but it cannot be used to differentiate between serovars (Wollanke et al., 2018). A geographical map of leptospiral hotspots may be useful for predicting exposure risk in horses (Sauvage et al., 2018).

Treatment for ERU generally involves immunosuppression and anti-inflammatories, which is what most of the horses in the current study received (Deeg, 2008; Deeg et al., 2006a; Verma & Stevenson, 2012). Many horses in this study received a secondary form of treatment after the first visit either for leptospiral infection or secondary complications caused by uveitis. Prophylactic treatment with doxycycline or minocycline may be useful early in the course of disease; however, this is controversial. Even with inflammation from uveitis causing increased drug penetration within the eye, it may not reach therapeutic levels (Gilmour et al., 2005). In the same study, oral doxycycline was not found within the eye at a standard 10 mg/kg bwt per os q. 12 h dose (Gilmour et al., 2005). We recommend treating with minocycline or doxycycline in horses with active uveitis and confirmed *Leptospira* infections (≥1:400) via MAT of serum or presence in the urine via PCR. Pars plana vitrectomy (PPV) is a popular treatment for ERU in Europe and is reported to be successful at preventing future uveitis flares (Tömördy et al., 2010). Horses should still undergo titre testing of the AH and vitreous prior to PPV, as well as aqueous humour PCR testing (Tömördy et al., 2010). Although PPV may be an effective method at preventing future ERU flares in some horses, it is still an invasive procedure associated with postoperative complications. Pars plana vitrectomy should be avoided in ERU horses with negative or low leptospiral intraocular titres as subconjunctival cyclosporine implants may be a safer effective alternative (Voelter et al., 2020). The goal of this study was to make suggestions for the early detection of leptospirosis and so that less invasive interventions may be implemented.

It is not currently known the percentage of horses that are chronic leptospirosis carriers or the percentage of those that clear the infection and do not develop ERU (Deeg, 2008). Treatment focused on immune-modulatory mechanisms to combat the cyclic nature of immune flares in ERU may prove useful in cases where leptospiral infection is established. Another avenue of future research can investigate retinal protective mechanisms as ERU autoantibodies attack the retinal tissues (Deeg, 2008; Deeg et al., 2006a; Verma & Stevenson, 2012). Prevention of autoantigen attack and cytokine production like INF-λ should be investigated to preserve the blood-retinal barrier and reduce inflammation (Deeg, 2008; Regan et al., 2012; Saldinger et al., 2020). Mesenchymal stem cell therapy can alter immune responses and may be useful to target intraocular inflammation (Saldinger et al., 2020; Sherman et al., 2017). Newer treatments such as low-dose gentamicin have been suggested but focus on prevention of flares and leptospiral infection may be more impactful for the control and preservation of vision (Fischer et al., 2019; Voelter et al., 2020).

The current relationship between leptospiral vaccines and leptospiral infection rate is unknown. Current vaccines for leptospirosis in horses commonly only include *L. pomona*. Our findings suggest that there are more pathogenic strains of leptospirosis regarding ERU like *L. bratislava*, *L. grippotyphosa* and *L. hardjo*. This hypothesis has also been proposed in the UK, as the prevalence of ERU seems to be higher in the USA than in the UK (Matthews et al., 1987). Horses living in wet areas or areas with a lot of wildlife should be vaccinated after proper screening for ERU. One horse in this study was suspected to develop ERU shortly after being vaccinated with a *Leptospira* vaccine. This is likely due to previously being infected by *Leptospira* as ERU from leptospirosis is not thought to occur on first exposure. However, it has been reported that a vaccine could have a protective effect on increasing length of time to an ERU flare but not the progression of disease (Rohrbach et al., 2005). Testing horses that are most at risk is advised before vaccinating as immune stimulation could cause an ERU flare. Further research should be aimed at investigating the relationship between ERU and vaccination status in horses. Perhaps vaccination should occur early in the horse's life before they are exposed to *Leptospira* as there may be a risk in vaccinating an infected horse.

In conclusion, our findings demonstrate that there is an association between leptospiral testing, younger horses and ocular posterior segment clinical disease. With this information, treatment and testing guidelines can be recommended to owners and veterinarians. Our findings support the need for further research directed at immune-modulatory treatments and vaccine development for ERU and leptospirosis.

Limitations

Limitations of this study are partly due to it being retrospective as information is obtained from medical and communication records. Due to NCSU-VHC being a referral hospital, it is likely that horses were lost to follow-up after their ERU flare was stabilised for treatment at home by the owner or with the referring veterinarian. NCSU-VHC may also be unintentionally screening for more advanced cases of ERU as a referral hospital, as some milder forms are likely treated by primary care veterinarians. Reliance on owners for accurate history on progression of signs, number of previous flares and severity of previous flares is challenging and this information was not always available. *Leptospira* can take a few days to

appear in serum, so some of the data here may be underestimating the number of affected horses that are not in the acute phase of disease. The home environment and housing of horses were not consistently asked at presentation, which could influence leptospiral infection risk. Different serovars that were not included in the laboratory testing may also be a contributing factor for ERU.

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Conflicts of interest

No conflicts of interest have been declared.

Author contributions

Both authors contributed to the study design, study execution, data analysis and interpretation, preparation of the manuscript and final approval of the manuscript.

Ethical animal research

Retrospective study – no specific ethical approval needed.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Summary of treatments administered by horse for the 53/63 (84%) horses diagnosed with ERU at the initial visit and any recheck visits. The treatments are broken down by topical treatments, systemic treatments, and other treatments including implants, injections, and enucleation. There were 52 horses treated at the first visit and 41 horses treated at a recheck visit. Horses could receive more than one treatment

Supplementary Item 2: Summary of initial ophthalmic exam findings in eyes diagnosed with ERU (n = 78). The table is organised from anterior to posterior findings. Not all eyes could be evaluated for posterior disease due to anterior clinical signs obscuring examination. This is demonstrated by the decrease in total number of eyes (n) evaluated posteriorly. Anterior clinical signs were present in 75/78 (96.15%) of eyes evaluated and posterior clinical signs were present in 37/59 (62.71%) of eyes able to be evaluated. Average aqueous flare score was 1.25+ out of 4